(2)

Institute Report No. 296

Mutagenic Potential of Triethyleneglycol Dinitrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

Steven K. Sano, BA, SGT, USA
and
Don W. Korte, Jr., PhD, MAJ, MSC

GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

September 1988

Toxicology Series: 113

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. ***(AR 360-5)***

for Richard A. Kishinoto 28 Sept 88

Edwin S. Beatrice (date)
COL, MC
Commanding

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 296	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Genetic Toxicology Branch, Division of Toxicology; 6b. OFFICE SYMBOL (If applicable): SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION: US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5010
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62720A; PROJECT NO. 835; TASK NO. AB; WORK UNIT ACCESSION NO. DA 303913

11. TITLE (Include Security Classification): Mutagenic potential of triethyleneglycol dinitrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test

12. PERSONAL AUTHOR(S): Steven K. Sano and Don W. Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM 1/7/85 TO 1/25/85	1988 September	20

16. SUPPLEMENTARY NOTATION: Toxicology Series 113

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Mutagenicity, Genetic toxicology, Ames Test, TRIETHYLENEGLYCOL DINITRATE, TEGDN, Propellant - (JES)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The mutagenic potential of TRIETHYLENEGLYCOL DINITRATE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 µl/plate to 0.0016 µl/plate both in the presence and absence of metabolic activation. The test compound was not mutagenic under conditions of this test.

micro Liters

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Edwin S. Beatrice, COL, MC	22b. TELEPHONE (Include Area Code): 415-561-3600; 22c. OFFICE SYMBOL: SGRD-UL-Z

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE: UNCLASSIFIED

ABSTRACT

The mutagenic potential of TRIETHYLENEGLYCOL DINITRATE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 µl/plate to 0.0016 µl/plate in both the presence and absence of metabolic activation. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, TRIETHYLENEGLYCOL DINITRATE, TEGDN, Propellant

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLBO

GLP STUDY NUMBER: 84048

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: Steven K. Sano, BA, SGT, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: TRIETHYLENEGLYCOL DINITRATE (TEGDN)

INCLUSIVE STUDY DATES: 7-25 January 1985

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of TRIETHYLENEGLYCOL DINITRATE (LAIR Code TA044) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

CPT John W. Harbell, PhD, MSC, and Mr. John Dacey provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP study number 84048 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 28 July 88

DON W. KORTE JR, PhD / DATE
MAJ, MSC
Study Director

Conrad Wheeler 19 July 88

CONRAD WHEELER, PhD/ DATE
DAC
Analytical chemist

Steven Kazuo Sano 5MAR86

STEVEN K. SANO, BA / DATE
SGT, USA
Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA

22 September 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance Statement

1. This is to certify that the protocol for GLP Study 84Ø48 was reviewed on 4 December 1984.

2. The institute report entitled "Mutagenic Potential of Triethyleneglycol Dinitrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 113, was audited on 12 May 1987.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

TABLE OF CONTENTS

TABLE OF CONTENTS

Mutagenic Potential of TRIETHYLENEGLYCOL DINITRATE in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test--Sano and Korte

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Toxicology Branch, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity test, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs. This report contains the results of a study to assess the mutagenic potential of TEGDN in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect those compounds which are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (2).

Objective of the Study

The objective of this study was to determine the mutagenic potential of TRIETHYLENEGLYCOL DINITRATE (LAIR Code TA044) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIAL AND METHODS

Test Compound

Chemical Name: TRIETHYLENEGLYCOL DINITRATE

LAIR Code Number: TA044

Physical State: yellow oil

Source: Naval Ordnance Station
Indian Head, MD

Storage: TRIETHYLENEGLYCOL DINITRATE was received and assigned the LAIR Code number TA044. The test compound was stored at room temperature (21°C) until use.

Chemical Properties/Analysis: Data characterizing the chemical composition and purity of the test material are presented in Appendix A.

Test Solvent

The test compound and the positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO).

Chemical Preparation

On the day before dosing, 500 µl of the test compound was measured into a sterile vial and again stored at room temperature. On the day of dosing, the 500-µl sample was dissolved in 9.4 ml of grade I dimethyl sulfoxide to achieve a 5% (v/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538, obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (3).

Test Format

TRIETHYLENEGLYCOL DINITRATE was evaluated for mutagenic potential according to the methods of Ames *et al* (4). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (3).

Toxicity Tests: Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of TRIETHYLENEGLYCOL DINITRATE ranging from 1.6×10^{-3} µl/plate to 5 µl/plate and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate.

Mutagenicity Test: The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (5). The water used in this medium and in all reagents came from a Polymetric model 200-3 Water Purifier (Sunnyvale, CA). Plates were incubated upside down in the dark, at 37°C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames *et al* (4). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The integrity

of the different *Salmonella* strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer of the cell wall is present.

-Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in the TA98 and TA100 strains.

-Lack of growth (inhibition) following exposure to ultraviolet light indicates the absence of the DNA excision-repair mechanism.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control, but may be tested with several. These compounds (benzo[a]pyrene, 2-aminofluorene, 2-aminoanthracene and N-methyl-N'-nitro-N-nitrosoguanidine) were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and the known mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (6), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Deviations from the Protocol/SOP

None.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

On 16 January 1985, the toxicity of TRIETHYLENEGLYCOL DINITRATE was determined (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the highest dose showed neither a decrease in the number of macrocolonies nor an observable reduction in the density of the background lawn, indicating no chemical toxicity. Therefore, the highest dose selected for the mutagenicity test was 5 µl/plate.

Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 23-25 January 1985 (Table 2). TRIETHYLENEGLYCOL DINITRATE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3).

A copy of the raw data is included in Appendix B.

DISCUSSION

Certain test criteria must be satisfied before the Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alteration in the LP (lipopolysaccharide) layer, and deficiency in DNA excision-repair. Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of the Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, TRIETHYLENEGLYCOL DINITRATE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose-response over three dose concentrations and a revertant colony count at least two times (TA98, TA100) or three times (TA1535, TA1537, TA1538) the spontaneous revertant colony count (6). TRIETHYLENEGLYCOL DINITRATE did not induce the requisite dose response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that TRIETHYLENEGLYCOL DINITRATE is not mutagenic when evaluated in the Ames Test.

TABLE 1: TOXICITY DETERMINATION FOR TEGDN (TA044)

GLP STUDY NUMBER: 84048 16 JAN 85 PERFORMED BY: SANO

TA 100 REVERTANT PLATE COUNT

TEST COMPOUND CONCENTRATION	MEAN (1SD)	BACKGROUND LAWN*
NEGATIVE CONTROL	95 (4.6)	NR
5.0 µl/plate	99 (13.4)	NL
1.0 µl/plate	96 (17.2)	NL
0.2 µl/plate	107 (17.6)	NL
0.04 µl/plate	85 (8.3)	NL
0.008 µl/plate	110 (2.1)	NL
0.0016 µl/plate	97 (6.0)	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	TA 100*
HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG (13mm)
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

* NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity
NR=Not Recorded

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF TEGDN (TA044)

GLP STUDY NUMBER: 84048 7-25 JAN 85 PERFORMED BY: SANO

STRAIN VERIFICATION

OBSERVATIONS*

STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET (zone size)	CONTROL
TA98	NG	G	NG	NG(14mm)	NG
TA100	NG	G	NG	NG(10mm)	NG
TA1535	NG	NG	NG	NG(12mm)	NG
TA1537	NG	NG	NG	NG(13mm)	NG
TA1538	NG	NG	NG	NG(10mm)	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

* NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity

TABLE 3: MUTAGENICITY ASSAY FOR TEGDN (TA044)

REVERTANTS/PLATE (MEAN ±1SD)

COMPOUND	DOSE/PLATE	TA98		TA100	
		WITHOUT S-9			
START NEG CONTROL	0.0 µg	16	±4.0	88	±10.2
MNNG†	2.0 µg	-	-	1017	±167.8
MNNG†	20.0 µg	-	-	-	-
TA044	5 µl	14	±6.6	42	±10.2
TA044	1 µl	24	±5.6	78	±5.5
TA044	0.2 µl	20	±4.7	109	±3.8
TA044	0.04 µl	22	±4.7	76	±12.5
TA044	0.008 µl	27	±7.0	84	±11.3
TA044	0.0016 µl	24	±3.6	95	±20.2
END NEG CONTROL	0.0 µg	15	±5.0	108	±7.9
		WITH S-9			
START NEG CONTROL	0.0 µg	27	±2.1	86	±15.1
2-AA†	2.0 µg	976	±366.9	1635	±30.4
2-AF†	2.0 µg	444	±135.5	533	±144.6
BP†	2.0 µg	180	±29.5	509	±19.7
TA044	5 µl	23	±1.5	95	±14.0
TA044	1 µl	20	±4.0	88	±20.5
TA044	0.2 µl	24	±3.6	72	±8.4
TA044	0.04 µl	21	±1.5	57	±11.6
TA044	0.008 µl	26	±7.0	101	±30.0
TA044	0.0016 µl	27	±2.6	121	±14.8
END NEG CONTROL	0.0 µg	24	±3.5	106	±8.0

† MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; 2-AA = 2-aminoanthracene; 2-AF = 2-aminofluorene; BP = benzo[a]pyrene.

TABLE 3 (cont.): MUTAGENICITY ASSAY FOR TEGDN (TA044)

REVERTANTS/PLATE (MEAN ±1SD)

COMPOUND	DOSE/PLATE	TA1535		TA1537		TA1538	
		WITHOUT S-9					
START NEG CONTROL	0.0 µg	13	±1.5	8	±2.0	23	±3.6
MNNG†	2.0 µg	-	-	-	-	-	-
MNNG†	20.0 µg	1812	±217.2	-	-	-	-
TA044	5 µl	23	±4.9	5	±0.6	30	±4.6
TA044	1 µl	18	±4.2	4	±3.8	31	±10.4
TA044	0.2 µl	21	±3.5	4	±2.3	29	±3.0
TA044	0.04 µl	25	±2.3	8	±0.6	44	±2.0
TA044	0.008 µl	19	±2.6	4	±1.5	35	±6.0
TA044	0.0016 µl	17	±5.8	5	±2.1	35	±5.7
END NEG CONTROL	0.0 µg	21	±2.3	6	±1.0	29	±5.9
		WITH S-9					
START NEG CONTROL	0.0 µg	13	±2.5	6	±3.8	28	±4.0
2-AA†	2.0 µg	-	-	245	±56.5	1286	±102.0
2-AF†	2.0 µg	-	-	-	-	559	±82.5
BP†	2.0 µg	-	-	44	±3.6	88	±4.0
TA044	5 µl	21	±4.6	6	±1.2	25	±5.0
TA044	1 µl	22	±3.1	7	±1.5	19	±8.0
TA044	0.2 µl	17	±3.1	4	±3.5	30	±3.6
TA044	0.04 µl	12	±3.1	6	±1.2	19	±7.0
TA044	0.008 µl	17	±4.2	4	±2.1	27	±6.2
TA044	0.0016 µl	18	±4.4	5	±1.2	30	±1.2
END NEG CONTROL	0.0 µg	14	±1.7	4	±1.0	25	±4.0

† MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; 2-AA = 2-aminoanthracene; 2-AF = 2-aminofluorene; BP = benzo[a]pyrene.

CONCLUSION

TRIETHYLENEGLYCOL DINITRATE was evaluated for mutagenic potential in the Ames Test, in both the presence and absence of metabolic activation, and did not produce a positive mutagenic response under conditions of this study.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.

2. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the *Salmonella*/Mammalian microsome test: Test of 300 chemicals. Proc Natl Acad Sci USA 1975;72:5135-5139.

3. Ames *Salmonella*/Mammalian Microsome Mutagenesis Test. LAIR Standard Operating Procedure OP-STX-1, Letterman Army Institute of Research, Presidio of San Francisco, California, 15 November 1983.

4. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with *Salmonella*/Mammalian microsome mutagenicity test. Mutat Res 1975;31:347-364.

5. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 1956;218:97-106.

6. Brusick D. Genetic Toxicology. In: Hayes AW, ed. Principles and Methods of Toxicology. New York: Raven Press, 1982: 223-272.

APPENDIX A: CHEMICAL DATA

Chemical Name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, dinitrate

Alternate Chemical Names: Triethyleneglycol dinitrate (TEGDN), NOSET-A

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code No.: TA044

Chemical Structure:

$$O_2N\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}NO_2$$

Molecular Formula: $C_6H_{12}N_2O_8$

Molecular Weight: 240

Physical State: Yellow oil

Density: (g/cm^3): 1.32*

Source: Naval Ordnance Station, Indian Head, MD, 20640

Lot No.: 130-84

---

* Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p17.

APPENDIX A (cont.): CHEMICAL DATA

Analytical data: The compound chromatographed as a single peak (retention time 5.8 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% methanol; flow rate 0.9 ml/min, detection wavelength, 215 nm.[†] No impurities were detectable by NMR.[‡] NMR (80 MHz, $CDCl_3$): 3.65 (S, 4H, $-CH_2-O-CH_2CH_2-O-CH_2-$), 3.72-3.84 (Complex multiplet, 4H, terminal methylene groups). IR (KBr): 2900, 1630, 1280, 1130, 1030, 910, 860 cm.[§]

Stability: The compound was received as a 10% solution in ethanol. Periodic analysis of this solution by HPLC has shown no evidence of decomposition to date (4 months).[†] NMR analysis demonstrated that the neat compound is stable for at least 1 month.[‡]

[†] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p26-30, 42-43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[‡] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p63. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[§] Ibid. p64.

APPENDIX B: INDIVIDUAL PLATE SCORES

TRIETHYLENEGLYCOL DINITRATE (TA044)
TOXICITY TEST
TA100

DOSES	5.0 µl/p	1.0 µl/p	0.2 µl/p	0.04 µl/p
PLATE 1	114	116	87	78
PLATE 2	89	84	114	94
PLATE 3	93	89	120	82
background lawn	normal lawn	normal lawn	normal lawn	normal lawn

DOSES	0.008 µl/p	0.0016 µl/p	NEG CONTROL
PLATE 1	111	97	90
PLATE 2	112	91	98
PLATE 3	108	103	98
background lawn	normal lawn	normal lawn	not recorded

APPENDIX B (cont.): INDIVIDUAL PLATE SCORES

NEGATIVE CONTROLS FOR MUTAGENICITY TESTS
TRIETHYLENEGLYCOL DINITRATE (TA044)

COMPOUND	DOSE	TA98	TA100	TA1535	TA1537	TA1538
WITHOUT S-9						
NEG CONTROL (START RUN)	0.0 µg/plate	20	91	15	8	20
		12	77	12	10	27
		16	97	13	6	22
NEG CONTROL (END RUN)	0.0 µg/plate	10	99	20	7	22
		20	114	20	5	31
		14	111	24	6	33
WITH S-9						
NEG CONTROL (START RUN)	0.0 µg/plate	28	74	13	2	29
		25	81	11	9	32
		29	103	16	8	24
NEG CONTROL (END RUN)	0.0 µg/plate	20	98	12	4	21
		27	114	15	5	24
		24	106	15	3	29

APPENDIX B (cont.): INDIVIDUAL PLATE SCORES

POSITIVE CONTROLS FOR MUTAGENICITY ASSAY
TRIETHYLENEGLYCOL DINITRATE (TA044)

COMPOUND†	DOSE	TA98	TA100	TA1535	TA1537	TA1538
2-AA	2.0 µg/plate	1352	1600		288	1283
		619	1647		181	1185
		956	1657		266	1389
2-AF	2.0 µg/plate	370	369			600
		600	586			613
		361	643			464
BP	2.0 µg/plate	206	500		41	93
		148	532		48	86
		186	496		43	86
MNNG	2.0 µg/plate		926			
			1211			
			915			
MNNG	20.0 µg/plate			2047		
				1619		
				1769		

† MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; 2-AA = 2-aminoanthracene; 2-AF = 2-aminofluorene; BP = benzo[a]pyrene.

APPENDIX B (cont.): INDIVIDUAL PLATE SCORES

TRIETHYLENEGLYCOL DINITRATE (TA044)
MUTAGENICITY TESTS

WITHOUT S-9

COMPOUND	DOSE	TA98	TA100	TA1535	TA1537	TA1538
TA044	5 µl/plate	15	38	20	5	26
		20	54	29	5	35
		7	35	21	4	29
TA044	1 µl/plate	23	74	21	2	38
		30	75	19	1	19
		19	84	13	8	36
TA044	0.2 µl/plate	18	113	18	3	29
		16	106	21	3	26
		25	107	25	7	32
TA044	0.04 µl/plate	20	70	26	8	44
		27	90	22	7	46
		18	67	26	8	42
TA044	0.008 µl/plate	20	91	20	6	36
		28	90	21	4	29
		34	71	16	3	41
TA044	0.0016 µl/plate	20	103	10	3	41
		25	72	20	7	30
		27	110	20	6	33

APPENDIX B (cont.): INDIVIDUAL PLATE SCORES

TRIMETHYLOLETHANE TRINITRATE (TA044)
MUTAGENICITY TESTS

WITH S-9

COMPOUND	DOSE	TA98	TA100	TA1535	TA1537	TA1538
TA044	5 µl/plate	25	105	26	7	30
		23	79	17	5	20
		22	101	20	5	26
TA044	1 µl/plate	20	67	19	7	27
		24	108	25	5	18
		16	89	21	8	11
TA044	0.2 µl/plate	21	62	14	8	26
		28	77	20	2	31
		23	76	18	2	33
TA044	0.04 µl/plate	21	69	15	5	26
		19	55	13	5	12
		22	46	9	7	19
TA044	0.008 µl/plate	33	77	12	5	20
		25	108	18	6	32
		19	117	20	2	29
TA044	0.0016 µl/plate	25	128	20	6	31
		26	131	21	4	31
		30	104	13	6	29

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (27)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314